Effect of Dietary Unsaturated Fatty Acids on Senile Amyloidosis in Senescence-Accelerated Mice

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Effects of dietary oils on aging were investigated in senescence-accelerated mice. For 26 weeks, mice were fed purified diets containing 4% olive oil, safflower oil, perilla oil, or fish oil. Serum total, high-density lipoprotein cholesterol, and apolipoprotein A-II (ApoA-II) were significantly lower in the fish oil group than in the perilla oil group, and these were significantly lower than in the olive oil or safflower oil group. The olive oil and safflower oil groups had significantly fewer ApoA-II amyloid fibril (AApoAII) deposits and anti–single-strand DNA (ssDNA) antibodies than the fish oil or perilla oil group, and the fish oil diet induced significantly more AApoAII deposits and anti-ssDNA antibodies than did the perilla oil diet. Survival decreased earlier in the fish oil group than in the other groups (as seen in the survival curve). The results suggest that greater the degree of unsaturation of dietary fatty acids, greater is the tendency for accelerated senescence.

Key Words: Dietary oil—Aging—Amyloidosis—Senescence-accelerated mice (SAM)

A murine model of accelerated senescence was established by Takeda and colleagues (1): senescence-accelerated mouse (SAM) P strains and control SAMR mouse strains with normal aging characteristics. The SAMP1 strain is characterized by earlier onset and irreversible advancement of senescence (manifested by clinical signs and gross lesions following the normal process of development), age-associated disorders, senile amyloidosis, and age-related appearance of immune dysfunctions (2). Senile amyloid protein deposits in the SAMP strain increase with advancing age in all tissues except bone and brain parenchyma (3,4). Biochemical studies have shown that apolipoprotein A-II (ApoA-II), a major apoprotein of plasma high-density lipoprotein (HDL), is a serum precursor of murine senile amyloidosis (5,6), and whole ApoA-II is deposited as amyloid fibrils (AAapoAII) without degradation (7,8). Previous studies have shown that SAMP1 mice that were fed energy-restricted diets failed to develop amyloidosis and that senescence grading scores indicated a retarded decline of immunologic function with aging; consequently, these mice had a prolonged mean life span (9,10).

Several reports claim that dietary lipids can alter the outcome of diseases with an inflammatory or immune dysfunction component. The incidence of amyloidosis rose in patients who consumed substantially increased amount of saturated fat in their diets (11). The induction of Amyloid A (AA)-type amyloidosis in young CBA/J mice was enhanced when diets enriched with coconut oil were substituted with diets containing n-3 or n-6 polyunsaturated fatty acids (PUFAs; 12). In an Alzheimer mouse model, n-3 PUFA intake reduced the amyloid burden (13). Studies have shown that a fish oil diet improves rheumatoid arthritis in human beings (14) and suppresses autoimmune disease in NZB/NZW F1 mice (15).

Previous studies have shown that a diet heavy in butter enriched with saturated fatty acids, or a fish oil diet with n-3 PUFAs, advanced the severity of senile amyloidosis with aging, whereas safflower oil enriched with n-6 PUFAs had the opposite effect, that is, it alleviated the severity of the age-related disease (16). These findings are contrary to the popular hypothesis that n-3 PUFAs are beneficial to health. In the present study, we investigated whether treatment with long-chain or short-chain n-3 PUFAs is associated with accelerated senescence in SAMP1 mice, and whether mono-unsaturated fatty acids (MUFA) have the same effect as n-6 PUFAs. We studied the effects of olive oil (rich in the MUFA; oleic acid), safflower oil (rich in n-6 PUFA; linoleic acid), perilla oil (rich in the short-chain n-3 PUFA; α-linolenic acid), and fish oil (rich in long-chain n-3 PUFA; eicosapentenoic acid [EPA] and docosahexanoic acid [DHA]) on SAMP1 mice on the basis of signs of accelerated senescence, the senescence grading score, the severity of amyloidosis, and immune dysfunction with aging.

Methods

Animals and Diets

SAMP1@Umz mice were obtained from our breeding colony where they were bred under conventional conditions. The colony was maintained by sister–brother breeding of SAMP1 mice, generously provided by Dr Takeda of...
Table 1. Fatty Acid Composition of the Oils Used in This Study (in g/100 g total fatty acids)

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Olive Oil</th>
<th>Safflower Oil</th>
<th>Perilla Oil</th>
<th>Fish Oil</th>
<th>CD</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:00</td>
<td>10.5</td>
<td>6.5</td>
<td>6.4</td>
<td>7.8</td>
<td>15.4</td>
</tr>
<tr>
<td>18:00</td>
<td>2.6</td>
<td>2.2</td>
<td>1.6</td>
<td>1.7</td>
<td>1.8</td>
</tr>
<tr>
<td>18:1(n-9)</td>
<td>73.7</td>
<td>19.4</td>
<td>17.8</td>
<td>9.9</td>
<td>22.7</td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>9.9</td>
<td>69.1</td>
<td>14.3</td>
<td>29.7</td>
<td>49.4</td>
</tr>
<tr>
<td>18:3(n-3)</td>
<td>0.7</td>
<td>0.4</td>
<td>58.5</td>
<td>0.3</td>
<td>3.4</td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td></td>
<td></td>
<td></td>
<td>19.0</td>
<td>1.8</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td></td>
<td></td>
<td></td>
<td>7.8</td>
<td>1.7</td>
</tr>
</tbody>
</table>

Note: CD = commercial diet. All the oils were stored at 4°C. Additional vitamin E (α-tocopherol) was added to the oils as an antioxidant so that the final concentration in the mixed diets was 0.018%.

Kyoto University (1). Six-week-old male SAMPI mice were fed diets containing olive oil, safflower oil, perilla oil, or fish oil. The major fatty acids in these diets are shown in Table 1. The experimental diets consisted of (by weight) 25% casein, 38% cornstarch, 25% sucrose, 2% cellulose powder, 5% mineral mixture, 1% vitamin mixture, and 4% fat (10). Diet solutions were stored at −35°C to prevent fatty acid oxidation. Diets containing less than 30 mEq/kg peroxide were routinely used. Each group of mice was fed a specific diet for 26 weeks. In addition, 8-month-old SAMPI male mice, which were fed a commercial diet (CD; CE-2; NIHON CLEAR, Tokyo, Japan) from the age 4 weeks, were used. The CD was made up of 25.2% crude protein, 50.2% carbohydrate, 4.4% fat, 4.4% fiber, and 7.0% ash.

The mice were housed at three or four per cage, allowed free access to food and tap water, and maintained in a temperature-controlled environment (24 ± 2°C) with a 12-hour light–dark cycle. All mice were maintained according to the policies and recommendations of the Koshien University Animal Care and Use Committee.

Evaluation of Senescence (senescence grading score)

This system was designed to represent changes in the behavior and appearance of the mice related to the aging process (17). The 11 categories in which changes were measured included behavior (reactivity and passivity), skin and hair (glossiness, coarseness of coat, hair loss, and ulcers), eyes (periphthalmic lesions, cataracts, corneal ulcers, and corneal opacity), and others (e.g., lordokyphosis). Each category had five grades of intensity of characteristics or changes. Each mouse was examined by inspection and palpation, and the sum of the scores of all 11 categories was recorded.

Measurement of Serum Lipids and Lipoprotein

At 32 weeks, mice were starved for about 15 hours before blood samples were collected, which were obtained by cardiac puncture following light anesthesia with ether. The serum was then stored at −35°C until analysis. Total cholesterol, HDL cholesterol, and triglyceride concentrations in serum were measured using cholesterol C tests, HDL cholesterol C tests, and triglyceride tests, respectively (Wako Pure Chemical Industries, Osaka, Japan). Serum concentrations of ApoA-I and ApoA-II were measured using an immunoblotting method (18). The serum was subjected to a 15%–20% gradient sodium dodecyl sulfate polyacrylamide minigel electrophoresis. After electrophoresis, the samples were electroblotted onto polyvinylidene difluoride membranes (Bio-Rad Laboratories, Richmond, CA). The presence of ApoA-I and ApoA-II was detected using the avidin-biotinylated horseradish peroxidase complex method after the membranes were incubated with monospecific rabbit anti-mouse ApoA-I and ApoA-II antisera (a dilution of 1:4000). Relative concentrations of ApoA-I and ApoA-II were determined by comparing the intensity of bands of these apoproteins with those of the internal control (i.e., purified mouse ApoA-I and ApoA-II protein) using a densitron information display system.

Histological Examination

The abdominal skin, liver, spleen, heart, and stomach of each mouse were fixed in 10% (v/v) neutral buffered formalin, embedded in paraffin, cut into 4-μm sections, and stained with hematoxylin and eosin or alkaline Congo red (19). Green birefringence under a polarizing microscope indicated the presence of amyloid deposition. The peroxidase–antiperoxidase method (20) with anti-AApoAII (5) was used to identify different types of amyloid fibril proteins in the immunohistochemical study. The intensity of AApoAII amyloid deposition was determined semiquantitatively using the amyloid index (AI) as a parameter (21). The AI is the average degree of AApoAII deposition in the stained sections of the organs examined, which were graded from 0 to 4.

Measurement of Anti–Single-Stand DNA Antibody Level

Serum concentrations of anti–single-strand DNA (ssDNA) antibody were measured using a mouse anti-ssDNA enzyme-linked immunosorbent assay kit (AKRSD-051; Shibayagi, Gunma, Japan).

Statistical Analysis

Survival curves were compared using the logrank test with the use of StatView J-4.5 software. Analysis of variance and a post hoc Tukey’s test were used to compare physical and biochemical characteristics between the four diet groups. The grading scores and amyloid indexes of the various organs in the four groups were compared using the Mann–Whitney U test. In addition, multiple regression analysis was performed using the senescence grading scores as the dependent variable and the scores for each component as independent variables. SPSS version 15.0 (SPSS Inc, Chicago, IL) was used for the analyses. Significance was established when p was less than .05.
Results

Growth Curve and Survival

No statistically significant differences were noted in food intake (g/mouse/d) among the mice fed each of the four oil diets: olive oil diet (7.4 ± 1.1), safflower oil diet (6.9 ± 0.9), perilla oil diet (7.1 ± 1.4), and fish oil diet (6.8 ± 0.5). The mice on olive oil or safflower oil diet showed similar changes in survival curves and body weight from 6 weeks to 8 months of age (Figure 1). Survival in the perilla oil diet group decreased moderately after 6 months of age, but no weight loss occurred. In contrast, survival curve for the fish oil diet group declined rapidly after 4 months of age and body weight loss occurred after 5 months of age. A comparison of survival curves revealed a significant difference between the fish oil group and the other three groups (p < .01). No difference was observed between the olive oil, safflower oil, and perilla oil groups.

Senescence Grading Scores

The various grading scores of mice at the age of 5 and 8 months reflecting senescence are shown in Figure 2. A significant increase in total grading scores from the ages of 5 to 8 months was observed in the groups dieting on safflower oil, perilla oil, and fish oil (p < .05) but was not seen in the group on olive oil diet. The total grading scores for the mice fed perilla oil or fish oil were significantly higher than those for the olive oil or safflower oil diet group at 8 months of age (p < .05), and the scores for the fish oil group tended to be higher than those for the perilla oil group (p < .10).

Serum Lipid Concentrations

The serum total cholesterol was highest in the olive oil group, followed by the safflower oil, perilla oil, and fish oil groups. Significant differences were observed between groups (p < .05; Table 2). HDL cholesterol was significantly lower in the fish oil and perilla oil groups than in the olive oil or safflower oil diet group and was significantly lower in the fish oil group than in the perilla oil group (p < .01). Triglyceride concentrations were significantly lower in the perilla oil and fish oil groups than in the olive oil and safflower oil groups (p < .01).

Serum Apolipoprotein Concentrations

Both serum ApoA-I and ApoA-II concentrations were significantly lower in the perilla oil and fish oil groups than in the olive oil and safflower oil groups (p < .01; Table 3); specifically, serum ApoA-II concentrations were 50% (perilla oil diet) and 25% (fish oil diet) lower in the olive oil and safflower oil groups, respectively. The ratio of ApoA-I to ApoA-II was markedly higher in the perilla oil and fish oil groups than in the olive oil and safflower oil groups (p < .01).

Amyloid Deposition

The severity of amyloid deposition, based on the AI, in organs from mice fed the various dietary oils is shown in Table 4. The AIs indicated that amyloid deposition was highest in the fish oil group (p < .05). Amyloid deposition in skin was significantly higher in the perilla oil group than in the olive oil and safflower oil diet groups; similarly, the AI was relatively higher too (p < .05). Although there was a trend for mice in the safflower oil group to have a higher AI than mice in the olive oil group, the difference was not significant.

Serum Anti-ssDNA Antibody Concentrations

As shown in Table 5, serum anti-ssDNA antibody concentrations were significantly higher in the perilla oil and fish oil groups than in the olive oil and safflower oil groups (p < .01), and significantly higher in the fish oil group than in the perilla oil group (p < .05).

Factors Related to Senescence Grading Score

Six variables—body weight (r = -.48), AI (r = .62), serum total cholesterol (r = -.82), serum HDL cholesterol (r = -.83), serum ApoA-II (r = -.74), and anti-ssDNA antibody (r = .77)—correlated significantly with senescence grading scores. A multivariate stepwise regression analysis was...
performed using the scores for these variables as independent variables. This analysis showed that serum HDL cholesterol concentrations ($\beta = −0.825$) influenced senescence grading scores ($p < .01$; Figure 3).

**Discussion**

Dietary fat supplementation in SAMP mice has been shown to alter the fatty acid composition of phospholipids from brains and improve learning performance (22) and to alter serum HDL lipids composition and promote amyloid depositions (16) in a tissue-specific manner. These findings suggest that the fatty acid composition of tissues is related to dietary fatty acid composition in SAMP mice. In the present study, we observed that SAMP1 mice fed an MUFA-rich (olive oil) diet or an n-6 PUFA–rich (safflower oil) diet had fewer age-related disorders than those fed n-3 PUFA–rich diets. The fish oil diet, which was enriched in long-chain n-3 PUFAs, caused remarkable age progression compared with the perilla oil diet, which was enriched in shorter chain n-3 PUFAs. Similarly, the fish oil diet also advanced age-related immune dysfunction. Although the survival curves by 8 months of age did not differ significantly between the olive oil, safflower oil, and perilla oil groups, further continuous observation is recommended.

The physiological effects of n-3 PUFA–rich oil, especially fish oil, are well documented; however, our findings in SAMP1 mice are contradictory. Fish oil rich in n-3 PUFAs rather than in n-6 PUFAs had a beneficial effect on inflammatory properties and life span in autoimmune-prone mice (23–25). Fish oil, which contains vitamin E, modulates the concentration of cytokines and thereby delays the onset of autoimmunity (26). Although n-3 and n-6 dietary lipids are susceptible to lipid peroxidation, fish oil has a beneficial effect. It regulates the activity of antioxidant enzymes in NZB/NZW mice (27,28). PUFAs of both the n-6 and the n-3 families are clinically useful in autoimmune inflammatory disorders, but the precise mechanisms responsible are not well understood (29).

It has been shown that longer living animal species have lower concentrations of unsaturated fatty acids, especially low concentrations of highly unsaturated fatty acids such as

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**Table 2. Serum Lipids Concentrations (in mg/dL) in SAMP1 Mice Fed Diets Containing Olive Oil, Safflower Oil, Perilla Oil, or Fish Oil for 26 Weeks**

<table>
<thead>
<tr>
<th>Diet</th>
<th>$n$</th>
<th>Total Cholesterol</th>
<th>HDL Cholesterol</th>
<th>Triglyceride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olive oil</td>
<td>14</td>
<td>165.3 ± 6.1*</td>
<td>74.5 ± 8.0</td>
<td>95.8 ± 35.3</td>
</tr>
<tr>
<td>Safflower oil</td>
<td>12</td>
<td>135.9 ± 22.2</td>
<td>79.2 ± 14.8</td>
<td>83.0 ± 21.6</td>
</tr>
<tr>
<td>Perilla oil</td>
<td>13</td>
<td>84.1 ± 11.3*</td>
<td>47.2 ± 9.9†</td>
<td>46.0 ± 16.2²</td>
</tr>
<tr>
<td>Fish oil</td>
<td>7</td>
<td>38.7 ± 20.9*</td>
<td>11.5 ± 2.7³</td>
<td>51.4 ± 15.9³</td>
</tr>
<tr>
<td>CD (age 32–36 wk)</td>
<td>10</td>
<td>90.2 ± 11.3</td>
<td>31.6 ± 13.1</td>
<td>69.0 ± 19.6</td>
</tr>
</tbody>
</table>

*Notes: CD = commercial diet; HDL = high-density lipoprotein; SAMP1 = senescence-accelerated mouse P1. Data are means ± SD. CD (CE-2; NIHON CLEAR) was fed to SAMP1 mice throughout their lives.

* $p < .05$ vs safflower oil, perilla oil, and fish oil.
† $p < .05$ vs olive oil, safflower oil, and fish oil.
² $p < .05$ vs olive oil and safflower oil.
³ $p < .01$ vs olive oil, safflower oil, and perilla oil.

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**Table 3. Serum Concentrations of ApoA-I and ApoA-II (in mg/dL) in SAMP1 Mice Fed Diets Containing Olive Oil, Safflower Oil, Perilla Oil, or Fish Oil for 26 Weeks**

<table>
<thead>
<tr>
<th>Diet</th>
<th>$n$</th>
<th>ApoA-I</th>
<th>ApoA-II</th>
<th>ApoA-I/ApoA-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olive oil</td>
<td>14</td>
<td>101.5 ± 14.1</td>
<td>32.0 ± 4.2</td>
<td>3.17 ± 0.3</td>
</tr>
<tr>
<td>Safflower oil</td>
<td>12</td>
<td>86.9 ± 20.4</td>
<td>28.5 ± 9.6</td>
<td>3.19 ± 0.6</td>
</tr>
<tr>
<td>Perilla oil</td>
<td>12</td>
<td>62.6 ± 19.1†</td>
<td>18.7 ± 6.2²</td>
<td>4.01 ± 1.7†</td>
</tr>
<tr>
<td>Fish oil</td>
<td>7</td>
<td>34.6 ± 6.2²</td>
<td>7.8 ± 2.5¹</td>
<td>4.39 ± 1.3¹</td>
</tr>
<tr>
<td>CD (age 32–36 wk)</td>
<td>9</td>
<td>109.2 ± 22.7</td>
<td>24.3 ± 2.5</td>
<td>4.77 ± 1.0</td>
</tr>
</tbody>
</table>

*Notes: ApoA-I = apolipoprotein A-I; CD = commercial diet; SAMP1 = senescence-accelerated mouse P1. Data are means ± SD. CD (CE-2; NIHON CLEAR) was fed to SAMP1 mice throughout their lives.

* $p < .05$ vs olive oil, safflower oil, and fish oil.
† $p < .05$ vs olive oil and safflower oil.
² $p < .01$ vs olive oil, safflower oil, and perilla oil.
DHA (22:6n-3), than species with shorter life span (30). Saturated fatty acid and MUFA acyl chains are essentially resistant to peroxidation, whereas PUFAs are damaged by it. Greater the degree of polyunsaturation of PUFAs, the more prone it is to peroxidative damage. DHA is eight times more prone to peroxidation than linoleic acid and 320 times more susceptible to peroxidation than MUFA (31). Mice of the SAMP strains, which are markedly short lived, have greater concentrations of the highly polyunsaturated peroxidation-prone fatty acids (22:6n-3 and 20:4n-6) and lower concentrations of the less peroxidation-prone PUFAs (18:2n-6) in their membranes than do mice of the SAMR strains, which have normal aging characteristics (32,33). However, SAMP mice have a lower resistance to oxidative stress than SAMR mice (34–36). In view of the changes in membrane composition and lipid peroxidation with age, the efficiency of the systems utilizing reactive oxygen species in tissues of the SAMP1 mice fed n-3 PUFA–rich oils, especially fish oil, which easily causes lipid oxidization, might be lower than that in other groups. However, it would be necessary to identify the fatty acid compositions in SAMP1 mice fed these dietary oils.

The SAMP1 mice fed on the n-3 PUFA–rich fish oil diet rather than the perilla oil diet had greater amyloid deposits than those fed on MUFA- or n-6 PUFA–rich diet; they also had low concentrations of serum ApoA-II. Rodent Abeta (1-42) exhibits oxidative stress properties similar to those of human Abeta (1-42 (37)). Although there is no evidence to suggest that ApoA-II aggregates to form amyloid fibrils, these changes can disrupt the structure of the membrane and alter its functional properties (e.g., susceptibility to peroxidative damage, fluidity, and distribution of lipid rafts) and might activate specific pathways involved in ApoA-II fibrilogenesis (38–40). Previous reports have shown that serum ApoA-II clearance accelerates with increasing age, and several organs trap more ApoA-II in old SAMP1 mice than in young mice (41). Increases in EPA and DHA in HDL resulting from an n-3 PUFA–rich diet (16) might further accelerate the clearance of serum ApoA-II.

A diet of olive oil appears to be very effective against progressive senescence in SAMP1 mice. Not surprisingly, the olive oil–rich Mediterranean diet is associated with healthier aging and increased longevity in human beings (42). Research in human beings has long yielded evidence of a protective effect of olive oil against cancer (43); in human beings and animals, olive oil has a higher antioxidant capacity and lower DNA damage with aging compared with safflower oil (44,45). A low concentration of unsaturated fatty acids, such as oleic acid, decreases cellular oxidative stress (30). In the present study, the olive oil diet tended to reduce amyloid deposition and immune dysfunction caused by aging more so than the safflower oil diet. Consequently,
increases in senescence scores with aging might be suppressed by olive oil consumption.

Concentrations of serum cholesterol in SAMP1 mice fed on olive oil or safflower oil increased to nearly the same concentrations as SAMR1 mice with normal aging (16). High concentrations of HDL and HDL cholesterol are associated with a lower total risk for mortality in older men (46,47); therefore, men and women considering cholesterol-lowering treatment should be aware of these findings (48). Higher concentrations of total cholesterol have also been shown to be associated with a decreased risk for incidental Alzheimer’s disease (49). Few reports exist on the injurious effects of lower serum cholesterol concentrations in animal models. The reason why high concentrations of serum total and HDL cholesterol protect against accelerated senescence in SAMP1 mice needs to be studied further. Regression analysis showed that senescence had a negative correlation with serum HDL cholesterol concentrations in SAMP1 mice. This finding indicates a close connection between advances in senescence and characteristics of senile amyloidosis in SAMP1 mice. In human studies, high concentrations of HDL cholesterol are associated with better functional performance in very old individuals (50). The senescence grading score system in SAMP strain mice was a reliable marker of frailty and poor prognosis.

Our findings of accelerated senescence scores, senile amyloid deposition, and immune dysfunction with aging in mice fed diets rich in n-3 PUFAs were related to the characteristic lipid or lipoprotein metabolism in SAMP1 mice. Conversely, we found that the dietary oils used in the present study, which are resistant to peroxidation, ameliorated the advancement of senescence in SAMP1 mice that had greater concentrations of lipid peroxides in their tissues (34). We emphasize the importance of genetic–dietary interactions controlling the lipid and lipoprotein metabolism. Additional studies are needed to clarify the relation between dietary fats and the progression of aging.

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